the BAC DNA from which they were prepared yielded the same results as the BAC DNA. See Applicants' specification, Examples 1 and 2. In addition, Applicants found that this method overcame two other technical challenges associated with arraying large DNA molecules. Specifically, the claimed method allows the production of target solutions that are representative of high molecular weight polynucleotides, but have a viscosity low enough for spotting on a substrate. In addition, the method enables the rapid, efficient, and inexpensive production of large quantities of DNA that is representative of the starting polynucleotides.

Claim 1 recites a "method for preparing amplification products useful for forming an array of polynucleotides that is representative of a plurality of first polynucleotides." The method employs "a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first [i.e., a starting] polynucleotide." Claim 1 recites "ligating adaptors to each end of the polynucleotide fragments to produce modified polynucleotide fragments, . . . and amplifying the modified polynucleotide fragments." According to the method, each sample is amplified and resuspended to form a target solution that is "representative of the corresponding first polynucleotide." Therefore, the plurality of target solutions produced by the method are representative of the plurality of starting polynucleotides. This feature of the invention facilitates meaningful comparison among hybridization signals produced when the target solutions are arrayed and hybridized with sample polynucleotides.

Claim 2 relates to applying the target solutions of claim 1 to one or more substrates to produce a polynucleotide array. Claim 3 relates to an embodiment of the method of claim 1 in which the double-stranded polynucleotide fragments are derived from a polynucleotide library. This embodiment enables the production of representative target solutions, e.g., where each solution corresponds to a clone in the library. Claim 20 recites an "array of polynucleotides that is representative of a plurality of first polynucleotides wherein said array is produced according to the method of Claim 2 and comprises at least 1000 amplification products in a 1 cm^2 region of substrate." Claim 21 recites a "plurality of target solutions produced according to the method of Claim 3." All of the remaining claims depend, directly or indirectly, from one of these claims. Thus, all of the pending claims incorporate the requirement for target solutions or array elements that are "representative of a plurality of first polynucleotides." See Claim 21.

35 U.S.C. § 102.

Claims 1, 3-13, and 21 were rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Smith (PCR Methods and Applications (1992) 2:21-27. Office Action, page 2. The rejection is respectfully traversed.

The Examiner states that "Smith teaches ligation-mediated PCR of restriction fragments from large DNA molecules." Office Action, page 2. The Examiner believes that "it is a property of Smith's target solution comprising amplification products, that this target solution would be suitable for application to a substrate" (*id.*, page 3), although the Examiner correctly states that "Smith does not teach using amplification products to generate an array" (*id.*, page 4). Nevertheless, the Examiner concludes that Smith anticipates the pending claims because the "claims recite 'preparing amplification products useful for forming an array" and "resuspending each amplification product to form a target solution suitable for application to a substrate'." *Id.*, page 3.

The Examiner's rationale does not take into account the fact that the method of claim 1 produces a plurality of target solutions, each of which is "***representative of the corresponding first polynucleotide***." As described in the specification, representative target solutions are conveniently produced by fragmenting a starting polynucleotide. Applicants' specification, page 10, lines 14-25. Applicants' specification states:

> Adaptors are ligated to each end of the polynucleotide fragments, which provides the fragments with common priming sites for amplification. Adaptors are typically designed to serve as efficient amplification primers so that unligated strands of the adaptors can be employed to amplify the sequences between the priming sites. This approach allows amplification of an polynucleotide without prior knowledge of the nucleotide sequence and allows the production of amplification products that are representative of the starting polynucleotide . . . .

Id.

By contrast, Smith discloses a "general method . . . for PCR amplification of ***single restriction fragments*** from large DNA molecules. Smith, abstract (emphasis added). Smith emphasizes the importance of the "ability to generate ***unique*** amplified fragments." Smith, page 23, col. 2. The experimental work disclosed in this article entailed the amplification of specific restriction fragments from restriction digested lambda DNA. See, e.g., Smith, page 22, col. 3 and Fig. 2 (showing that amplification produced a singe 163 bp fragment). In summing up, Smith states:

"This study demonstrates the applicability of ligation-mediated PCR to amplifying ***individual*** type IIS restriction fragments from large DNA molecules. Smith, page 24, col. 3 (emphasis added).

The Smith method seeks to amplify a specific fragment of a large DNA molecule, such as lambda DNA. This goal is diametrically opposed to that of the claimed method, which seeks to produce an amplification product that is representative of the entire starting polypeptide, not just a fragment. Thus, Smith's teaching regarding the amplification of a unique sequence within a larger molecule teaches directly away from the claimed method and compositions.

Smith also contemplates sequencing the unique sequences produced in this manner. Accordingly, Smith suggests that the "use of many different adapter-tags permits sample mixing for multiplex sequencing protocols to allow efficient chemical sequencing of these products." Smith, page 25, col. 3. It is not entirely clear how such a method would be carried out in practice. Moreover, after examining the theoretical feasibility of using this method for genomic sequencing, Smith concludes:

> [Twenty] gaps averaging 225 bp (accounting for 6% of the sequence) were evident. Most of these gaps could have been easily bridged in one step by primer walking. However, the task of generating the templates would have required 80 bubble ligations and 2880 adapter-tag ligations with 100% success in the PCR reactions. The limited experimental data presented in this paper suggests that an actual success rate would be lower. Nevertheless, this approach might still be useful.

Smith, page 25-26. Far from suggesting anything remotely like the claimed method, this statement indicates that a multiplex variation of the Smith method would not be practicable for producing an amplification product that is representative of the corresponding starting polynucleotide.

As the method of claim 1 produces target solutions wherein each "target solution [is] representative of the corresponding first polynucleotide" and claim 21 recites a "plurality of target solutions" that also have this feature, claims 1 and 21, as well as their dependent claims, are clearly distinct from the methods and compositions described in Smith. Accordingly, Applicants respectfully request withdrawal of the § 102 rejection of claims 1, 3-13, and 21.

35 U.S.C. § 103(a).

Smith and Brown

Claims 2, 14-16, and 20 were rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Smith in view of Brown et al. (USPN 5,807,522). Office Action, page 3. This rejection is respectfully traversed.

Claim 2 is a method claim depending from claim 1 and thus incorporates the requirement that the recited target solutions be "representative of the corresponding first polynucleotide." Claims 14-16 depend, directly or indirectly, from claim 2. Claim 20 recites an "array of polynucleotides that is representative of a plurality of first polynucleotides." As discussed above, Smith neither teaches nor suggests this element of the pending claims. Applicant submits that Brown, whether taken alone or in combination with Smith, fails to remedy this deficiency.

Brown is primarily concerned, not with the production of target solutions, but, as the Examiner recognizes, with a method and apparatus for applying target solutions to a substrate to fabricate microarrays. Office Action, page 4; Brown, abstract. Brown discloses the use of PCR to "randomly" amplify DNA for robotic spotting on substrates (Brown, col. 16, lines 9-22; col. 17, lines 46-55), but nothing in Brown teaches or suggests any measures that would ensure that the product of this amplification was representative of the corresponding starting polynucleotides. Accordingly, Brown is silent with respect to the method that Applicants employed to achieve this goal, which, as recited in claim 1, entails:

> a) providing a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first polynucleotide; [and]
>
> b) ligating adapters to each end of the polynucleotide fragments to produce modified polynucleotide fragments, wherein each adapter comprises a first strand and a second strand, the second strand having a region of substantial complementarity to a region of the first strand.

Brown's reference to "random" PCR amplification suggests that random primers were used in the amplification reaction. The products obtained using random primers are not generally "representative of the starting nucleic acid" because the efficiency of amplification of a given segment depends upon the ability of the primers that anneal to sequences flanking the segment to form stable hybrids. This capability is, in part, nucleotide sequence dependent. Random primers,

by definition, contain primers having different nucleotide sequences. Thus, in random primer-mediated amplification, given hybridization conditions will favor annealing of some primers over other primers, producing a distribution of sequences in the amplification product that is not representative of the starting polynucleotide.

As the Smith-Brown combination fails to teach or suggest any method of producing target solutions that are representative of the starting polynucleotides, much less the method recited in the pending claims, the claims are patentable over this combination. Withdrawal of the § 103 rejection of claims 2, 14-16, and 20 is therefore respectfully requested.

Smith, Brown, and Gordon

Claim 17 was rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Smith in view of Brown and further in view of Gordon et al. (USPN 5,601,980). Office Action, page 5. The rejection is respectfully traversed.

Claim 17 depends ultimately from claim 1 and recites a method "wherein the target solutions are robotically spotted on the substrate." Gordon was cited for the teaching of robotic spotting. *Id.* However, Gordon fails to provide the teaching of representative target solutions, which is missing from Smith and Brown. In addition, Gordon fails to teach or suggest any method for producing such solutions. Accordingly, the combination of Smith, Brown, and Gordon does not teach or suggest all of the elements of the claim1, which are incorporated into claim 17 by virtue of its dependence on claim 1. Applicants therefore respectfully request withdrawal of the 103 rejection of claim 17.

Smith, Brown, and Stimpson

Claim 18 was rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Smith in view of Brown and further in view of Stimpson et al. (Proc. Natl. Acad. Sci. USA (1995) 92:6379-6383). Office Action, page 5-6. The rejection is respectfully traversed.

Claim 18 depends ultimately from claim 1, which recites the use of adaptors in the claimed amplification reaction. Claim 18 recites that "at least one strand of the adapters includes an amino group." Gordon was cited as teaching "DNA chips (i.e., array[s]), which are constructed by using 3'-amino-labeled oligonucleotides." Office Action, page 6. However, Stimpson discloses that "DNA chips . . . were constructed by using presynthesized 3'-amine-labeled oligonucleotides." Stimpson, page 6380, col. 1. Stimpson thus fails to teach or suggest anything regarding an

amplification-based method for producing target solutions that are representative of corresponding starting polynucleotides. Stimpson thus does nothing to remedy the above-noted deficiencies of Smith and Brown. Accordingly, the combination of Smith, Brown, and Stimpson does not teach or suggest all of the elements of the claim1, which are incorporated into claim 18. Applicants therefore respectfully request withdrawal of the § 103 rejection of claim 18.

Smith and Cronin

Claims 19 and 22 were rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Smith in view of Cronin et al. (WO 97/43450). Office Action, page 6. The rejection is respectfully traversed.

Claim 19 depends from claim 1 and recites a method "wherein the target solutions comprise dimethyl sulfoxide [DMSO] at a concentration of about 20% by volume." Cronin was cited as teaching target solutions containing DMSO. Office Action, page 7. However, like the other references of record, Cronin fails to teach or suggest representative target solutions. In addition, Cronin fails to teach or suggest any method for producing such solutions. Cronin, like Stimpson, discloses arrays of synthetically produced oligonucleotides. Cronin, page 9, line 15 – page 10, line 29. Cronin also discusses the polynucleotides to be analyzed by hybridization to the array, which Cronin calls "target" polynucleotides. Cronin, page 8, lines 6-7. It should be noted that Cronin's target polynucleotides are the sample polynucleotides that bind to array elements, whereas the polynucleotides in the target solutions recited in the pending claims are useful as array elements. Cronin describes amplifying target (i.e., sample) polynucleotides by "PCR," but does not teach or suggest any measures to ensure that the amplified products are representative of the starting polynucleotides.

Thus, the Smith-Cronin combination fails teach or suggest all of the elements of the claim1, which incorporated into claim 19. Applicants therefore respectfully request withdrawal of the § 103 rejection of claim 19.

Claim 22 recites the "plurality of target solutions of Claim 21 wherein the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume. Claim 21 recites a "plurality of target solutions prepared according to the method of Claim 3." As claim 3 depends from claim 1, the target solutions of claim 22 are necessarily representative of the corresponding starting polynucleotides. Thus, for at least the reasons discussed above with respect to claim 19, the

Smith-Cronin combination does not teach or suggest all of the aspects of claim 22. As claim 22 is therefore patentable over this combination, withdrawal of the § 103 rejectiono of claim 22 is respectfully requested.

Conclusion

In view of the foregoing, Applicants believe that all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-8891.

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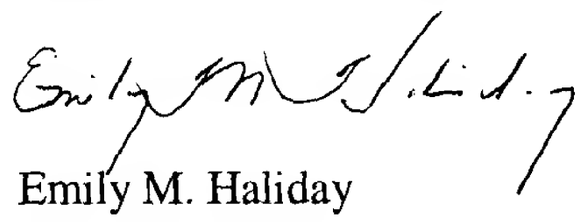
Respectfully submitted,



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APPENDIX A

"MARKED UP" CLAIMS ILLUSTRATING THE AMENDMENTS MADE TO THE CLAIMS OF 09/574386 WITH ENTRY OF THIS AMENDMENT

Amended claim(s):

1. A method for preparing amplification products useful for forming an array of polynucleotides that is representative of a plurality of first polynucleotides comprising:

a) providing a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first polynucleotide;

b) ligating adapters to each end of the polynucleotide fragments to produce modified polynucleotide fragments, wherein each adapter comprises a first strand and a second strand, the second strand having a region of substantial complementarity to a region of the first strand;

c) amplifying the modified polynucleotide fragments to produce an amplification product for each sample of polynucleotide fragments;

d) isolating each amplification product; and

e) resuspending each amplification product to form a target solution <u>representative of the corresponding first polynucleotide, wherein the target solution is</u> suitable for application to a substrate to produce an array of polynucleotides.

APPENDIX B

CLAIMS PENDING IN USSN 09/574386 WITH ENTRY OF THIS AMENDMENT

1. A method for preparing amplification products useful for forming an array of polynucleotides that is representative of a plurality of first polynucleotides comprising:

   a) providing a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first polynucleotide;

   b) ligating adapters to each end of the polynucleotide fragments to produce modified polynucleotide fragments, wherein each adapter comprises a first strand and a second strand, the second strand having a region of substantial complementarity to a region of the first strand;

   c) amplifying the modified polynucleotide fragments to produce an amplification product for each sample of polynucleotide fragments;

   d) isolating each amplification product; and

   e) resuspending each amplification product to form a target solution representative of the corresponding first polynucleotide, wherein the target solution is suitable for application to a substrate to produce an array of polynucleotides.

2. The method of Claim 1 additionally comprising applying the target solutions to one or more substrates, wherein each target solution is applied to a distinct location on one substrate and/or target solutions are applied to different substrates that are combined to produce an array of polynucleotides.

3. The method of Claim 1 wherein the double-stranded polynucleotide fragments are derived from a polynucleotide library.

4. The method of Claim 3 wherein the polynucleotide library is a genomic DNA library.

5. The method of Claim 3 wherein the polynucleotide library is a cDNA library.

6. The method of Claim 3 wherein the double-stranded polynucleotide fragments are derived from YAC, BAC, P1 or PAC clones.

7. The method of Claim 1 wherein the first polynucleotides each have a complexity of at least about 50 kilobases.

8. The method of Claim 1 wherein the first polynucleotides each have a complexity of at least about 100 kilobases.

9. The method of Claim 7 wherein the first polynucleotides each have a complexity of less than about 500 kilobases.

10. The method of Claim 1 wherein the double-stranded polynucleotide fragments are obtained using one or more restriction endonucleases.

11. The method of Claim 1 wherein the average length of the double-stranded polynucleotide fragments is less than about 5 kilobases.

12. The method of Claim 11 wherein the average length of the double-stranded polynucleotide fragments is less than about 2 kilobases.

13. The method of Claim 11 wherein the average length of the double-stranded polynucleotide fragments is greater than about 100 basepairs.

14. The method of Claim 2 wherein the average volume of each target solution applied to the substrate is less than about 2 nanoliters.

15. The method of Claim 14 wherein the average volume of each target solution applied to the substrate is equal to greater than about 0.002 nanoliters.

16. The method of Claim 2 wherein the array comprises at least 1000 amplification products in a 1 cm^2 region of substrate.

17. The method of Claim 2 wherein the target solutions are robotically spotted on the substrate.

18. The method of Claim 2 wherein at least one strand of the adapters includes an amino group.

19. The method of Claim 1 wherein the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume.

20. An array of polynucleotides that is representative of a plurality of first polynucleotides wherein said array is produced according to the method of Claim 2 and comprises at least 1000 amplification products in a 1 cm^2 region of substrate.

21. A plurality of target solutions prepared according to the method of Claim 3.

22. The plurality of target solutions of Claim 21 wherein the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume.